A Randomized Trial of Low Dose Cytarabine Alone or in Combination with Glasdegib in Patients With Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome

Jorge E. Cortes,
Department of Leukemia, University of Texas, MD Anderson Cancer Center, Houston, TX
Florian H. Heidel,
Otto-von-Guericke University Medical Center Magdeburg, Germany
Current affiliation: Internal Medicine II, University Hospital Jena, Germany
Andrzej Hellmann,
Department of Haematology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland
Walter Fiedler,
Department of Hematology and Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany
B. Douglas Smith,
Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD
Tadeusz Robak,
Department of Hematology, Medical University of Lodz, Lodz, Poland
Pau Montesinos,
Hospital Universitari i Politècnic La Fe, Valencia, Spain; CIBERONC, Instituto Carlos III, Madrid, Spain
Daniel A. Pollyea,
Division of Hematology, University of Colorado School of Medicine, Aurora, CO
Pierre DesJardins,
Hôpital Charles LeMoyne, Greenfield Park, Quebec, Canada
Oliver Ottmann,
Division of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, UK
Weidong Wendy Ma,
Pfizer Oncology, New York, NY
M. Naveed Shaik,
Pfizer Oncology, New York, NY
A. Douglas Laird,
Pfizer Oncology, New York, NY
Mirjana Zeremski,
Pfizer Oncology, New York, NY
Ashleigh O'Connell,
Pfizer Oncology, New York, NY
Geoffrey Chan, and
Pfizer Oncology, New York, NY
Michael Heuser,
Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Corresponding author: Dr Jorge E. Cortes. The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard Box 428, Houston, TX 77030, USA.

Telephone: +1 713-794-5783; Fax: +1 713-794-4297. Email: jcortes@mdanderson.org

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Running head: Glasdegib for AML/MDS pts not suitable for intensive chemotherapy

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Disclaimers:

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In full collaboration with the study investigators, the sponsor (Pfizer) contributed to the design and conduct of the study and the collection, management, analysis, and interpretation of study data. Employees of Pfizer who were involved in the design of the study, acquisition and analysis of data, and the preparation, review, and approval of the manuscript are included as authors. All authors had full access to all study data, were collectively responsible for the accuracy and completeness of the data and vouch for the fidelity of the trial to the protocol (available at xxxxxxxxxxxx). Manuscript development was led by the lead author. All authors contributed to drafting the manuscript and have reviewed and approved the final version for submission. Sponsor-funded medical writing support was provided by Engage Scientific Solutions of Envision Pharma Group.

2. Author Contributions

Study conception and design: Jorge E. Cortes, Weidong Wendy Ma, M. Naveed Shaik, Mirjana Zeremski, and Ashleigh O'Connell.

Development of methodology: Jorge E. Cortes, Weidong Wendy Ma, M. Naveed Shaik, Mirjana Zeremski, Ashleigh O'Connell, and Geoffrey Chan.

Data acquisition (acquired and managed patients, provided facilities, etc.): Jorge E. Cortes, Florian H. Heidel, Andrzej Hellmann, Walter Fiedler, B. Douglas Smith, Tadeusz Robak, Pau Montesinos, Daniel A. Pollyea, Pierre DesJardins, Oliver Ottmann, and Michael Heuser.

Analysis of data (e.g., statistical analysis, biostatistics, computational analysis): Jorge E. Cortes, Weidong Wendy Ma, M. Naveed Shaik, A. Douglas Laird, Mirjana Zeremski, Ashleigh O'Connell, and Geoffrey Chan.

Providing administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Weidong Wendy Ma, M. Naveed Shaik, A. Douglas Laird, Mirjana Zeremski, Ashleigh O'Connell, and Geoffrey Chan.

Study supervision: M. Naveed Shaik, Mirjana Zeremski, Ashleigh O'Connell, Geoffrey Chan, and Jorge E. Cortes.

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Weidong Wendy Ma: employee of and owns stock in Pfizer Inc.
M. Naveed Shaik: employee of and owns stock in Pfizer Inc.
A. Douglas Laird: employee of and owns stock in Pfizer Inc.
Mirjana Zeremski: employee of and owns stock in Pfizer Inc.
Ashleigh O'Connell: employee of and owns stock in Pfizer Inc.
Geoffrey Chan: employee of and owns stock in Pfizer Inc.
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ABSTRACT

PURPOSE—Glasdegib is a potent and selective oral inhibitor of the Hedgehog signaling pathway. This phase 2, randomized, open-label, multicenter study evaluated the efficacy of glasdegib plus low-dose cytarabine (LDAC) in patients with acute myeloid leukemia or high-risk myelodysplastic syndrome not suitable for intensive chemotherapy.

PATIENTS AND METHODS—Glasdegib 100 mg once daily was administered orally in 28-day cycles on a continuous basis; LDAC 20 mg was administered subcutaneously twice daily for 10 of every 28 days. Patients were stratified by cytogenetic risk factor (good/intermediate or poor) and randomized (2:1) to receive glasdegib/LDAC or LDAC. The primary endpoint was overall survival. Secondary objectives included other efficacy endpoints, safety, pharmacodynamics, and pharmacokinetics.

RESULTS—In all, 88 and 44 patients were randomized to receive glasdegib/LDAC and LDAC, respectively. Median (80% confidence interval [CI]) overall survival was 8.8 (6.9 to 9.9) months with glasdegib/LDAC and 4.9 (3.5 to 6.0) months with LDAC (hazard ratio, 0.51 [80% CI, 0.39 to 0.67], P = .0004). Fifteen (17.0%) patients in the glasdegib/LDAC arm and 1 (2.3%) patient in the LDAC arm achieved complete remission ($P < 0.05$). Most frequently (> 10%) reported nonhematologic grade 3/4 all-causality adverse events were pneumonia (16.7%) and fatigue (14.3%) with glasdegib/LDAC, and pneumonia (14.6%) with LDAC. Preliminary signs of clinical efficacy were evident across patients with diverse mutational profiles.

CONCLUSION—Glasdegib plus LDAC has a favorable benefit–risk profile and may be a promising treatment option for patients with acute myeloid leukemia or high-risk myelodysplastic syndrome not suitable for intensive chemotherapy.

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Key words: acute myeloid leukemia, glasdegib, Hedgehog, Smoothened inhibitor, myelodysplastic

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INTRODUCTION

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are clinically and genetically heterogeneous myeloid stem cell disorders with a median age at onset of about 67 years.[1] Older patients with AML or high-risk MDS have few treatment options and are often not eligible for intensive chemotherapy due to comorbidities and a higher incidence of high-risk biological features, which often lead to chemotherapy resistance.

This population is thus treated with less-aggressive therapies, including low-dose cytarabine (LDAC) and hypomethylating agents. However, studies with LDAC have demonstrated low response rates (7% to 18%), with median overall survival (OS) of 5 months in older patients.[2-7] With the hypomethylating agent decitabine, the response rate (18%) and median OS (7.7 months) were only slightly improved.[5] Therefore, novel therapeutic strategies are needed to achieve higher response rates, more durable responses, and improved survival in this hard-to-treat population.

The Hedgehog signaling pathway plays a key role in embryonic development and is typically silenced in adults.[8] Aberrant Hedgehog signaling has been implicated in hematologic malignancies and is critical for leukemia stem-cell survival and expansion.[9-11] Overexpression of Hedgehog pathway components was observed in chemotherapy-resistant myeloid leukemia cells, and pharmacologic inhibition of the Hedgehog pathway substantially enhanced the sensitivity to chemotherapy.[12] These findings provide the rationale for combining an inhibitor of Hedgehog pathway with chemotherapy.

Glasdegib is a potent and selective oral inhibitor of Hedgehog signaling through binding to Smoothened. In preclinical studies, glasdegib produced rapid and complete tumor regression as a single agent or in combination with chemotherapy, reduced expression of key leukemia stem-cell regulators, and decreased leukemia stem-cell populations in patient-derived AML cells.[13,14] Glasdegib monotherapy demonstrated preliminary clinical activity in phase 1 trials in patients with hematologic malignancies.[15,16] Therefore, glasdegib plus chemotherapy represents a mechanistically attractive treatment approach for patients with

AML and MDS.

A phase 1b/2, open-label, international, multicenter study evaluated safety and efficacy of glasdegib plus intensive chemotherapy (cytarabine and daunorubicin), LDAC, or decitabine in previously untreated patients with AML or high-risk MDS.[17,18] Here we describe results from the phase 2, randomized, open-label portion of the study that assessed the efficacy and safety of glasdegib plus LDAC (glasdegib/LDAC) versus LDAC in patients with AML or high-risk MDS who were not eligible for intensive chemotherapy.

METHODS

Patients

Eligible patients were aged ≥ 55 years with newly diagnosed, previously untreated AML or high-risk MDS, according to the World Health Organization (WHO) 2008 Classification.[19] For a diagnosis of high-risk MDS RAEB-2 (refractory anemia with excess blasts 2) the patient must have 10-19% bone marrow blasts. Patients had to have a known cytogenetic profile at study entry and considered not suitable for intensive chemotherapy, defined by one or more of the following criteria:[20] age ≥ 75 years; serum creatinine > 1.3 mg/dL severe cardiac disease (e.g., left ventricular ejection fraction < 45% by multi-gated acquisition or echocardiography at screening), or Eastern Cooperative Oncology Group performance status (ECOG PS) = 2. Patients with ECOG PS = 0 or 1 who met one or more other inclusion criteria listed above were also eligible (full inclusion criterial see Supplementary materials). Patients were excluded if they had acute promyelocytic leukemia, t(9:22) cytogenetic translocation, active malignancy, known active uncontrolled leukemia of the central nervous system, or prior treatment with Hedgehog inhibitor or other investigational agent for the treatment of an antecedent hematologic disease (more details see Supplementary materials).

Study Design and Treatment

In this phase 2 study (ClinicalTrials.gov, NCT01546038), patients were stratified by cytogenetic risk factor (good/intermediate, or poor) and randomized (2:1) to receive glasdegib/LDAC or LDAC. The

primary objective was OS. Secondary objectives included clinical efficacy endpoints, safety and tolerability, pharmacokinetics (PK), pharmacodynamics, and effect on corrected QT (QTc) interval.

Patients were classified as having poor-risk disease if they had one of the following cytogenetic features: inv(3), t(6;9), 11q23, –5, –5q, –7, abnormal (17p), or complex karyotype (≥ 3 clonal abnormalities). Patients with none of these features were classified as having good/intermediate-risk disease.[21,22] Glasdegib 100 mg once daily was administered orally in 28-day cycles on a continuous basis and LDAC 20 mg was administered subcutaneously twice daily for 10 days every 28 days. Patients remained on treatment until disease progression, unacceptable toxicity, or patient refusal. All patients were followed-up for post-treatment survival status for 4 years from randomization.

Patient randomization was obtained from the interactive voice response system. Masking was not applicable for this open-label study.

This study was conducted in compliance with the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice Guideline, and local regulatory requirements. The final protocol, amendments, and informed consent documents were approved by institutional review board or independent ethics committee at each investigational center. All patients provided informed consent.

Assessments

Efficacy

Response to treatment was assessed based on the International Working Group response criteria and WHO Guidelines for MDS and AML.[23,24] Immunophenotyping and cytogenetics were performed for all bone marrow samples (Supplementary materials).

Pharmacokinetics

Blood samples for PK analysis of glasdegib were analyzed for concentrations of glasdegib at Covance Bioanalytical Services, LLC (Indianapolis, IN, USA) using a validated, sensitive, and specific high-performance liquid chromatography–tandem mass spectrometric approach (Supplementary materials).

Safety

Safety assessments included adverse events (AEs), classified and graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0, laboratory evaluations, vital signs, physical examinations, and 12-lead electrocardiograms. Treatment duration and time of treatment exposure of glasdegib were also calculated (Supplementary materials).

Biomarker Analyses

Biomarker assessments included mutational status of the following genes: *CEBPA*, *DNMT3A*, *FLT3*, *IDH1*, *IDH2*, *KIT*, *KRAS*, *NPM1*, *NRAS*, *RUNX1*, *TET2,* and *WT1*. Whole blood samples from serial blood draws were analyzed for gene expression using TaqMan Low-Density Microarrays (TLDA) including 21 target genes implicated in Hedgehog pathway signaling and/or AML pathobiology (Supplementary materials).

Statistical Analyses

OS was defined as time from date of randomization to death from any cause. Patients not known to have died at the last follow-up were censored on the date they were last known to be alive. The reported median OS for LDAC was approximately 5 months[2-4] and the expected median OS for glasdegib/LDAC was 8 months, resulting in an expected HR = 0.625 (i.e., 60% improvement in OS). A total of 132 patients would be randomized at 2:1 ratio (i.e., 88 in the glasdegib/LDAC arm and 44 in the LDAC alone arm), of which 92 OS events observed would provide 80% power to detect the 60% improvement in OS at 1-sided significance level of 0.10 with an interim analysis (IA) for futility. The IA would occur when 46 OS events were observed (i.e., 50% information). Since the IA was for futility only, no alpha would be spent at the IA. The rho(1) spending function was used as the beta-spending function for futility at the IA. If exactly 46 OS events were observed at the IA, the futility boundary would be crossed if the observed HR >0.92. The futility boundary would be calculated accordingly using the chosen spending function and number of OS events actually observed at the IA..

Median OS and 80% CI were analyzed using the Kaplan–Meier method. A stratified log-rank test (one-sided $\alpha = 10\%$) was used to compare OS between the treatment arms. A Cox proportional hazard regression stratified by prognosis (good/intermediate vs. poor) was used to estimate the hazard ratio (HR) and 80% confidence interval (CI) of OS. Other efficacy endpoints were summarized descriptively and included complete remission (CR) and CR with incomplete blood count recovery (CRi). An additional efficacy endpoint for AML included morphologic leukemia-free state (MLFS). Additional efficacy endpoints for MDS included marrow complete remission (mCR) and partial remission. Safety data were summarized descriptively and included all randomized patients who received at least one dose of any of the study medications.

RESULTS

Patients

Overall, 132 patients were randomized to receive glasdegib/LDAC (n = 88) and LDAC (n = 44); among them, 84 and 41 patients received study treatments, respectively (Fig. 1). Patient demographic and baseline characteristics are summarized in Table 1. More male patients were included (69 in the glasdegib/LDAC and 26 in the LDAC group) and over half of the patients in each group (53 [60.2%] in the glasdegib/LDAC and 24 [54.5%] in the LDAC group) were older than 75 years. The median (range) number of cycles administered was 3 (1 to 35) with glasdegib/LDAC and 2 (1 to 9) with LDAC. Thirty-seven (44%) patients in the glasdegib/LDAC group and 15 (36.6%) patients in the LDAC group received follow-up systemic therapies after discontinuation of the study treatment. The majority of patients (34 in the glasdegib/LDAC and 14 in the LDAC group) received chemotherapy (Table S1).

Efficacy

Median follow-up for OS was 21.7 months with glasdegib/LDAC and 20.1 months with LDAC. The corresponding number of deaths were 68 (77.3%) and 41 (93.2%) patients. The main cause of death in both arms was disease progression (Tables S2 and S3). This translated into a median (80% CI and

95%CI) OS of 8.8 (6.9 to 9.9 and 5.0 to 11.7) months with glasdegib/LDAC and 4.9 (3.5 to 6.0 and 2.9 to 6.5) months with LDAC (HR, 0.51 [80% CI, 0.39 to 0.67; 95% CI, 0.34 to 0.77], $P = 0.0004$) (Fig. 2). The probability (80% CI and 95% CI) of being alive at 6 and 12 months, respectively, was 59.8% (52.6 to 66.3 and 48.6 to 69.4) and 39.5% (32.6 to 46.3 and 29.1 to 49.7) with glasdegib/LDAC versus 38.2% (28.6 to 47.7 and 23.8 to 52.4) and 9.5% (4.8 to 16.3 and 3.0 to 20.6) with LDAC. Results were similar when separate Cox proportional hazards model were estimated by cytogenetic risk (Fig. 3). In patients with AML (n = 116), median (80% CI and 95% CI) OS was 8.3 (6.6 to 9.5 and 4.7 to 12.2) months with glasdegib/LDAC and 4.3 (2.9 to 4.9 and 1.9 to 5.7) months with LDAC (HR, 0.46 [80% CI, 0.35 to 0.62; 95% CI, 0.30 to 0.72], $P = 0.0002$). In patients with MDS (n = 16), median (80% CI and 95% CI) OS was 10.9 (1.6 to 12.5 and 0.4 to 12.7) months with glasdegib/LDAC and 10.3 (6.0 to 11.7 4.9 to 15.1) months with LDAC (HR, 0.77 [80% CI, 0.37 to 1.63; 95% CI, 0.25 to 2.41], $P = 0.3280$).

Fifteen (17.0%) patients in the glasdegib/LDAC arm, and 1 (2.3%) patient in the LDAC arm achieved CR ($P < 0.05$, Table 2). In the glasdegib/LDAC arm, median (range) duration of response was 9.9 (0.03 to 28.8) months for patients with CR and 6.5 (0.03 to 28.8) months for patients with either CR, CRi, or MLFS. In the AML population, overall response rate (ORR; defined as CR plus CRi plus MLFS) was 26.9% with glasdegib/LDAC and 5.3% with LDAC. In the MDS population, ORR (defined as CR plus mCR) was 20.0% with glasdegib/LDAC and 0% with LDAC. Best overall response with other responses of interest for patients with AML and MDS are summarized in Tables S4 to S6.

Pharmacokinetics

Eighty-three and 69 patients in the glasdegib/LDAC arm were analyzed for PK concentration and PK parameters, respectively. Sixty-one of 69 patients evaluable for PK parameters were analyzed on Cycle 1 Day 10; of these, 41 did not receive cytochrome P450 (CYP)3A4 inhibitors concomitantly. Since CYP3A4 inhibitors have the potential to increase glasdegib plasma exposure, this group was considered to more accurately represent glasdegib plasma PK parameters for the 100-mg once-daily dose. These patients showed a somewhat lower exposure to glasdegib than those with exposure to CYP3A4 inhibitors.

Summary of glasdegib PK parameters for glasdegib/LDAC arm on Cycle 1 Day 10 is presented in Appendix Table 6 (online only). Median glasdegib plasma concentration–time profile on Cycle 1 Day 10 is presented in Appendix Fig. 1 (online only).

Safety

The median (range) treatment duration was 2.7 (0.1 to 31.9) months with glasdegib/LDAC and 1.5 (0.2 to 7.9) months with LDAC. The mean relative dose intensity (calculations see Supplementary materials) of glasdegib was 89.0% for the glasdegib/LDAC arm, and the mean relative LDAC dose intensity was 95.5% and 96.1% for the glasdegib/LDAC and LDAC arms, respectively.

The most frequently (> 5% of patients) reported nonhematologic grade 3/4 all-causality AEs with glasdegib/LDAC were pneumonia (16.7%), fatigue (14.3%), dyspnoea (7.1%), hyponatremia, sepsis, and syncope (6.0%, each), and pneumonia (14.6%) with LDAC (Table 3). The most frequently (> 5% of patients) reported nonhematologic grade 3/4 treatment-related AE (i.e., related to either LDAC and/or glasdegib) was fatigue (10.7%), which occurred in the glasdegib/LDAC arm (Appendix Table 7, online only).

Thirty (35.7%) and 19 (46.3%) patients permanently discontinued study treatments due to AEs, with nine (10.7%) and three (7.3%) patients discontinuing due to treatment-related (per investigator's assessment) AEs in the glasdegib/LDAC and LDAC arms, respectively. In the glasdegib/LDAC arm, 47 (56.0%) patients temporarily discontinued glasdegib and/or LDAC and 22 (26.2%) patients had study treatment dose reduced due to AEs. In the LDAC arm, 13 (31.7%) patients temporarily discontinued LDAC due to AEs. No dose reduction in LDAC due to AEs was reported.

Serious AEs were reported in 66 (78.6%) patients in the glasdegib/LDAC arm and 32 (78.0%) patients in the LDAC arm. The most frequently (≥ 15% of patients) reported serious AEs were febrile neutropenia (28.6% with glasdegib/LDAC, 17.1% with LDAC) and pneumonia (22.6% and 17.1%, respectively). In the glasdegib/LDAC arm, 3 (3.6%) patients had serious acute kidney injury (1 considered related to

glasdegib) and 1 (1.2%) patient had serious muscle spasms (considered related to glasdegib).

Nine and five patients in the glasdegib/LDAC and LDAC arms, respectively, had elevated liver function enzymes (total bilirubin, aspartate aminotransferase, and/or alanine aminotransferase). Most were grade 1/2; 3 patients in the glasdegib/LDAC arm had grade 3 (1 related and 2 unrelated to treatment). No patient had concurrent elevations of all enzymes and none was confirmed as Hy's law case.[25] No elevated liver enzymes led to permanent discontinuations of study treatments.

Abnormal Frederica's QTc (QTcF) findings, either mean QTcF > 480 ms and/or mean QTcF increase > 60 ms from baseline, occurred in nine patients treated with glasdegib/LDAC and five treated with LDAC. QTcF prolongation > 500 ms was less frequent with glasdegib/LDAC versus LDAC (6.0% vs 11.8%). Two patients temporarily discontinued treatment due to glasdegib-related electrocardiogram QT prolongation. Two patients had permanent dose reduction due to treatment-related electrocardiogram QT prolongation, 1 of which was related to glasdegib. No patients had Torsades de Pointes.

Biomarker Analyses

Eighty-eight patients were included in baseline mutational analyses of bone marrow and/or peripheral blood, including 61 patients who received glasdegib/LDAC and 27 patients who received LDAC. No significant differences in mutational frequency between responding and non-responding patients were evident (Fisher's exact test, $P > 0.05$ for each of the 12 genes analyzed). Responses were observed in patients bearing mutations in one or more of all 12 genes assessed except *KRAS*, but the small numbers preclude firm conclusions of associations of mutations in specific genes with response to therapy (Table S8). However, nonsignificant trends suggest that gene mutations associated with a favorable overall response to the combination treatment include *CEBPA*, *IDH1*, *NPM1*, *RUNX1*, and *TET2*, whereas gene mutations associated with an unfavorable overall response to the combination treatment include *DNMT3A*, *IDH2*, and *NRAS/KRAS*. Further, an ad-hoc exploratory analysis demonstrated no significant relationship to response for *TP53* mutational status (data not shown). Findings of RNA biomarker

analysis are described in the Supplementary materials.

DISCUSSION

This randomized phase 2 trial in patients with AML or high-risk MDS met its primary endpoint, as the addition of glasdegib to LDAC demonstrated statistically significant and clinically meaningful OS improvement. The patients treated with glasdegib/LDAC achieved a 49% reduction in the risk of death relative to LDAC (median 8.8 vs 4.9 months; HR, 0.51 [80% CI, 0.39 to 0.67; 95% CI, 0.34 to 0.77], P = 0.0004). In terms of the HR, improvement in OS was consistent across pre-specified subgroups by cytogenetic risk per interactive voice response system (IVRS) data, particularly in patients with good/intermediate cytogenetic risk. Furthermore, ORR with glasdegib/LDAC (26.9%) was higher compared with LDAC (5.3%). These results, together with the manageable safety profile, make the combination of Hedgehog inhibition with LDAC a compelling therapeutic approach particularly for patients with AML ineligible for intensive chemotherapy.

The subset of MDS patients treated with glasdegib/LDAC achieved a 22.8% reduction in the risk of death relative to LDAC, though the 80% CI around the OS HR encompassed one and the sample size was small. Considering that the analysis on patients with MDS was limited by the small sample size, more patients with MDS are being assessed (ClinicalTrials.gov, NCT02367456) to better understand the impact of glasdegib in MDS.

A median of 2 cycles of LDAC was administered, which was a shorter treatment period than the 4 cycles delivered in a prior study.[26] The open-label design of the current study may have contributed to this short treatment period with LDAC; however, this median number of cycles of LDAC was consistent with a most recent report by Dennis et al.[27] The CR rate in patients treated with glasdegib/LDAC (17.0%) was higher than in those treated with LDAC (2.3%). These results showed a lower CR rate with LDAC than previously published (7% to 22%), potentially because of the short treatment period (1.5 months) with LDAC in the current study.[2,5-7,26] The low CR rate in the LDAC arm in the current study may also be due

in part to the high proportion of patients with secondary AML who are known to be resistant to chemotherapy.[28] However, median OS with LDAC was similar to that observed in previous studies, suggesting that the control arm is representative of clinical expectations with this regimen.[6,29,30]

In the population treated with glasdegib/LDAC, glasdegib mean steady-state plasma PK parameters at 100 mg once daily were in agreement with the mean parameters observed in the phase 1b portion (Arm A) of the study.[17] The maximum plasma concentration (C_{max}) of glasdegib at 100 mg is adequate to cover the half maximal inhibitory concentration values required for inhibition of the Hedgehog pathway in vitro.[16] The similar means of C_{max} and AUC_{tau}, and the variability in these parameters (range, 44–61%) suggest that the intermittent use of moderate or strong CYP3A4 inhibitors is not associated with a large increase in glasdegib exposures on Cycle 1 Day 10. This indicates that CYP3A4 inhibitors may be used concomitantly as medically necessary.

Although comparison between trials should be considered with caution due to potential methodologic and other differences, median OS with glasdegib/LDAC compared favorably to previously reported outcomes with the combinations of LDAC/imatinib (4.6 months), LDAC/lintuzumab (4.7 months), LDAC/volasertib (4.8 months), or LDAC/volasertib (8.0 months).[6,29-31] Importantly, the addition of glasdegib to LDAC was generally well tolerated, with a manageable safety profile consistent with elderly patients receiving chemotherapy and toxicities reported for other marketed Smoothened inhibitors. The frequencies of alopecia, muscle spasms, and dysgeusia were numerically lower than what has been previously reported for Smoothened inhibitors.[32-34] The most common AEs occurring at higher rates in the glasdegib/LDAC versus LDAC arm were cytopenias and gastrointestinal events (mostly grade 1 to 2). Cytopenias were not accompanied by increases in sepsis or bleeding as compared with LDAC. Patients in the glasdegib/LDAC arm remained longer on treatment compared with the LDAC arm; therefore, it is possible the higher incidence of cytopenias in the glasdegib/LDAC arm was due to the longer duration of chemotherapy.

Preliminary signs of clinical efficacy were evident across patients with diverse mutational profiles, suggesting the potential for broad efficacy of glasdegib in combination with LDAC. However, no significant correlations were evident between mutational status of any of the individual 12 reported genes and clinical response. Nonsignificant trends suggesting association of gene mutations with response or lack of response were noted, but further research is required.

Reducing the incidence of disease progression to prolong survival remains the highest unmet medical need in the treatment of AML. Various agents targeting distinct pathways or markers are currently in development or have become available for clinical management of AML, such as azacitidine and venetoclax. Both drugs showed promising effects in treating AML as debulking agents.[35,36] via a different mechanism than that of the stem cell agent glasdegib. Preclinical data showed synergistic activity of SMO inhibitor (erismodegib) and azacitidine,[37] and in a Phase 1 trial glasdegib plus azacitidine showed evidence of clinical activity with no evidence of drug–drug interaction.[38] Aiming for an effective assessment of a novel therapy for patients with AML, a randomized, double-blind, multicenter, placebo controlled phase 3 trial (ClinicalTrials.gov, NCT03416179) of glasdegib in combination with intensive chemotherapy or azacitidine in patients with untreated AML is ongoing.

The addition of glasdegib to LDAC resulted in a favorable benefit-to-risk profile given the statistically significant and clinically meaningful improvement in OS compared with the standard therapy of LDAC and generally manageable toxicity. Therefore, the combination of glasdegib plus LDAC may represent a promising treatment strategy for patients with AML or high-risk MDS who are not suitable for intensive chemotherapy.

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Figure Legends

Figure 1: Patient disposition

Primary analysis data cut-off was 3 January 2017. Discontinuations were attributed to the last study treatment received. Treated was defined as patients who received at least one non-zero dose of glasdegib or LDAC.

Abbreviations: AE, adverse event; IVRS, interactive voice response system; LDAC, low-dose cytarabine; PK, pharmacokinetic(s).

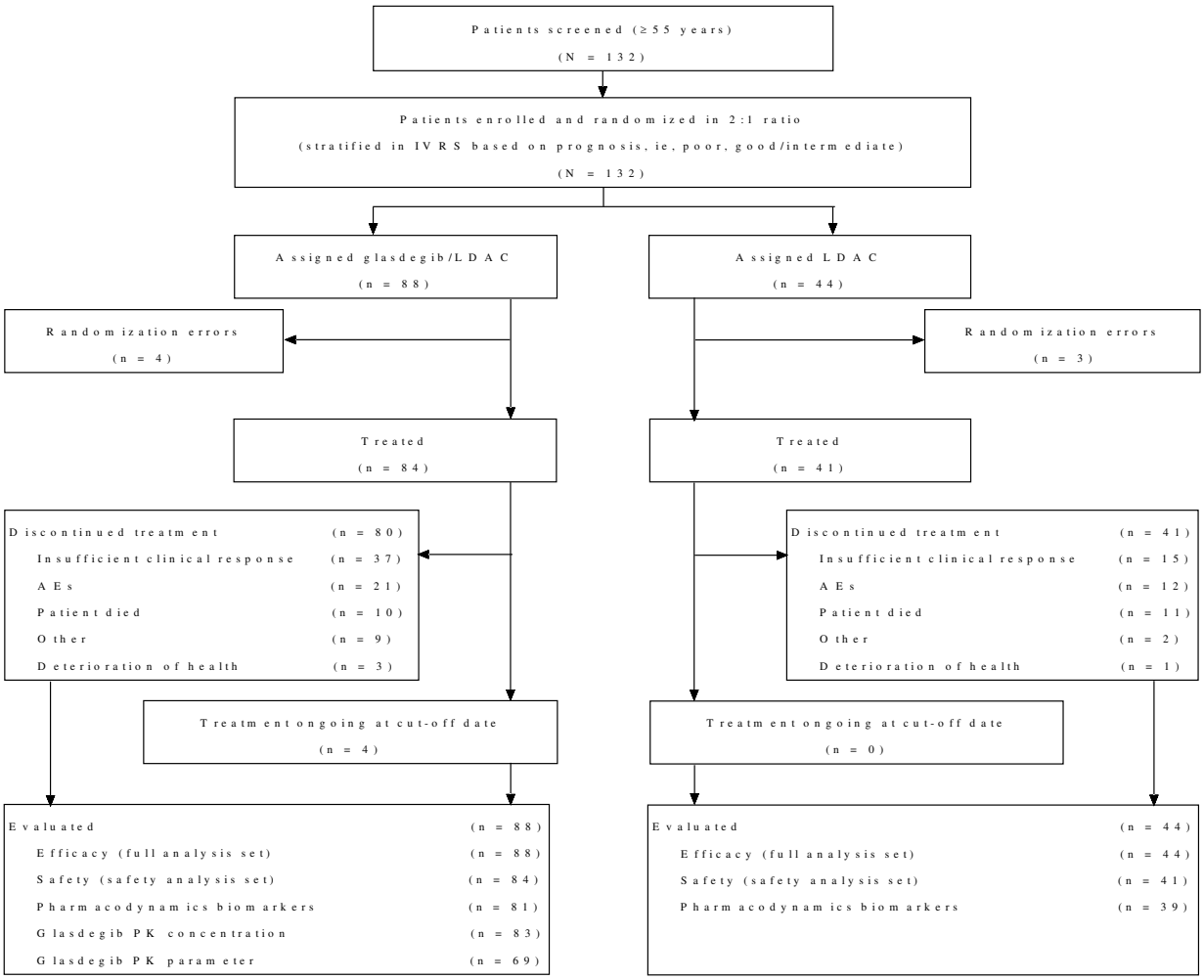
Figure 2: Kaplan–Meier estimate of overall survival, full analysis set

Abbreviations: CI, confidence interval; HR, hazard ratio; LDAC, low-dose cytarabine; OS, overall survival

Figure 3: Kaplan-Meier estimate of overall survival, full analysis set, in patients at (A) Good/intermediate cytogenetic risk and (B) Poor cytogenetic risk

Abbreviations: CI, confidence interval; HR, hazard ratio; LDAC, low-dose cytarabine; OS, overall survival

Fig 1. Patient disposition



Primary analysis data cut-off was 3 January 2017. The randomization errors in 7/132 patients (5%) were due to data entry errors in the IVRS. Discontinuations were attributed to the last study treatment received. Treated was defined as patients who received at least one non-zero dose of glasdegib or LDAC.

Abbreviations: AE, adverse event; IVRS, interactive voice response system; LDAC, low-dose cytarabine; PK, pharmacokinetic(s).

Fig 2. Kaplan-Meier estimate of overall survival, full analysis set

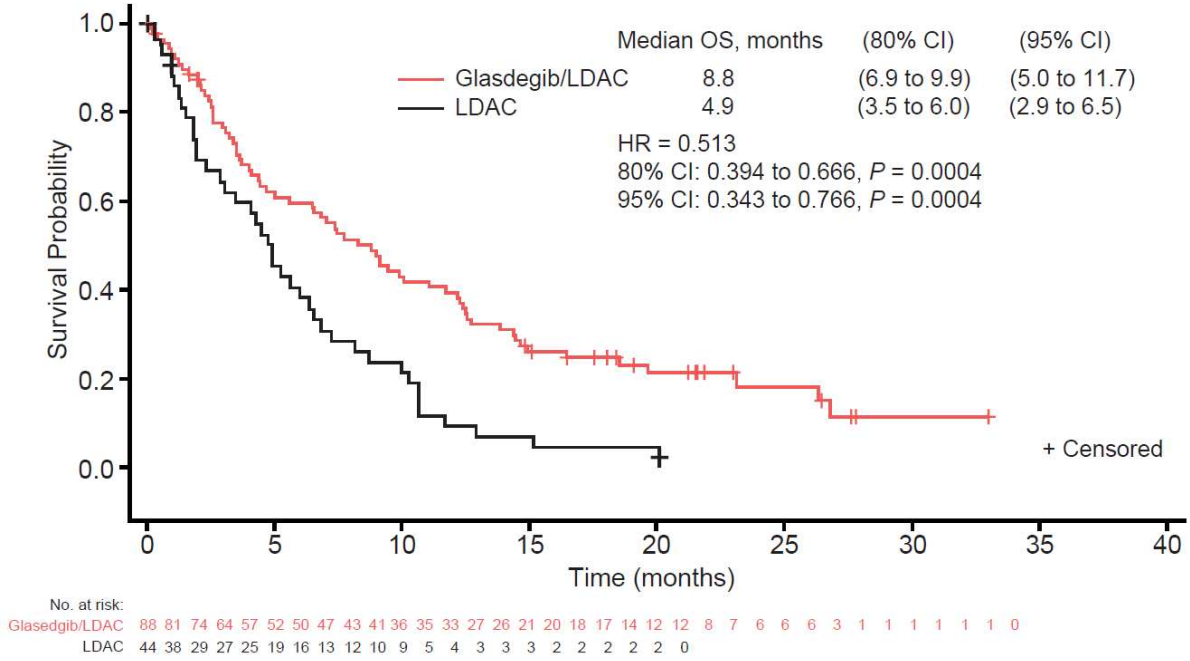
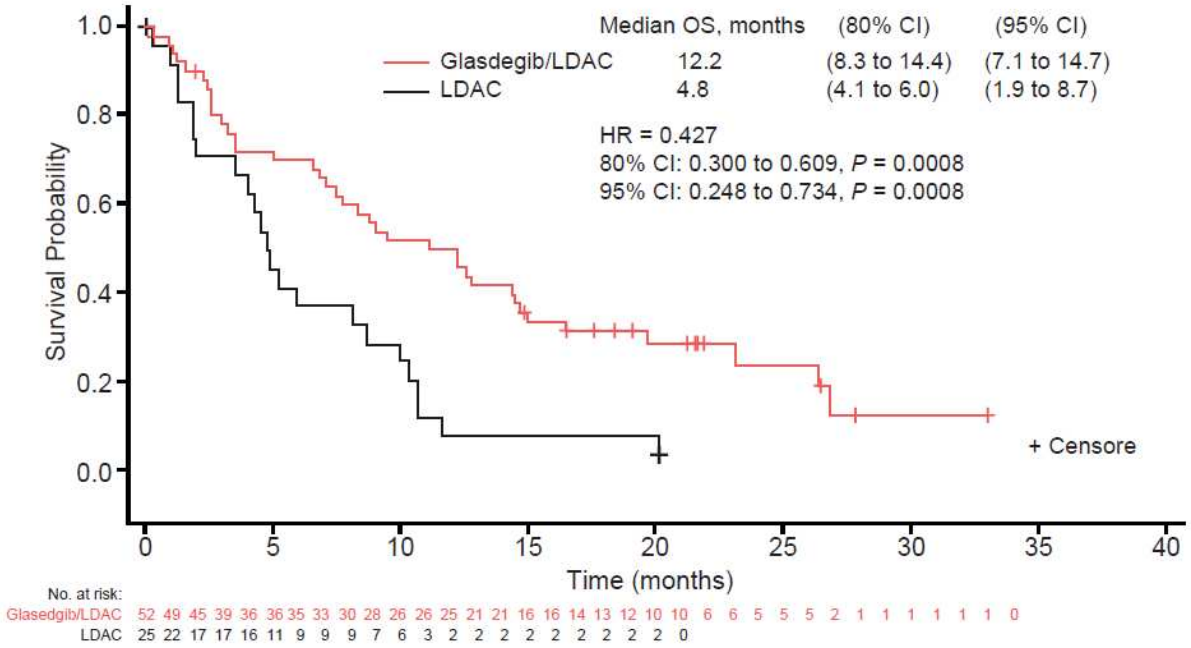


Fig 3. Kaplan-Meier estimate of overall survival, full analysis set

A. Good/intermediate cytogenetic risk



B. Poor cytogenetic risk

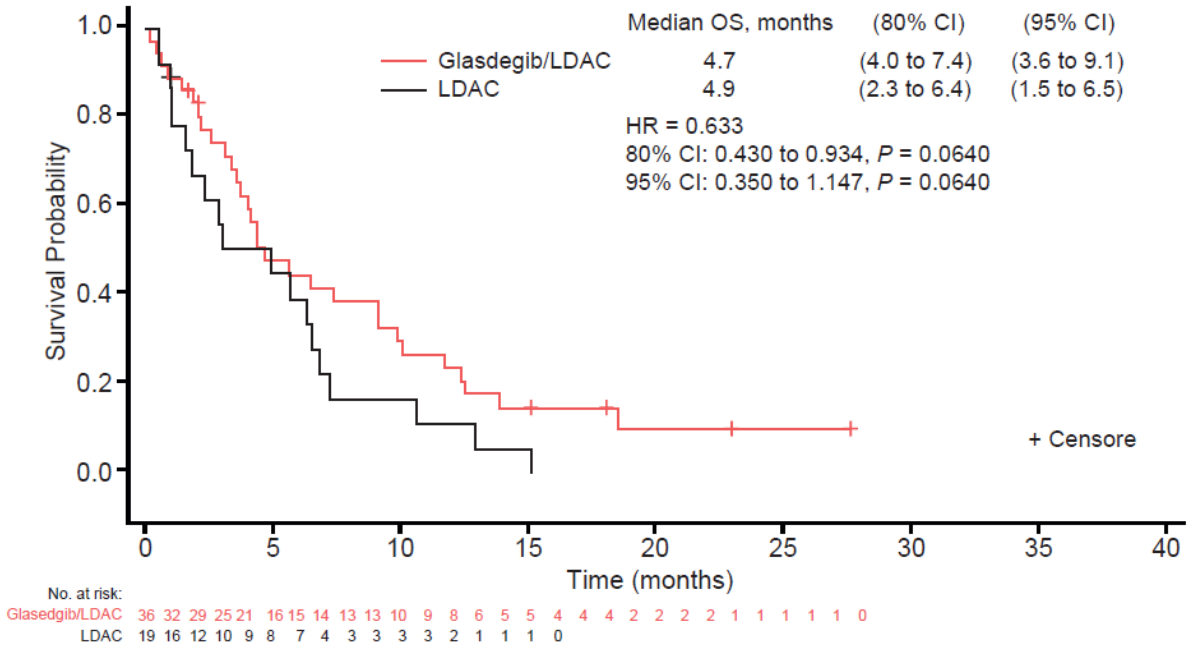


Table 1: Patient demographic and baseline characteristics

	Glasdegib 100 mg + LDAC N = 88	**LDAC N = 44**
Sex, n (%)		
Female	19 (21.6)	18 (40.9)
Male	69 (78.4)	26 (59.1)
Age (years)		
55–64, n (%)	2 (2.3)	1 (2.3)
65–74, n (%)	33 (37.5)	19 (43.2)
≥75, n (%)	53 (60.2)	24 (54.5)
Mean (SD)	76.2 (6.2)	74.5 (4.9)
Median (range)	77 (63–92)	75 (58–83)
Race, n (%)		
White	85 (96.6)	44 (100.0)
Black	1 (1.1)	0
Asian	2 (2.3)	0
Body mass index (kg/m^2)		
Mean (SD)	27.4 (4.2)	28.2 (5.5)
Range	17.5–41.9	20.0–48.2
Peripheral blood white cell count ($10^3/mm^3$)		
Median (range)	2.3 (0.6–64.0)	3.6 (1.1–45.2)
Diagnosis, n (%)		
AML	78 (88.6)	38 (86.4)
MDS	10 (11.4)	6 (13.6)
Bone marrow blasts (%)		
With AML, median (range)	41.0 (16.0–100.0)	46.0 (13.0–95.0)
With MDS, median (range)	14.0 (7.5–18.0)	16.0 (10.5–19.0)
Duration since histopathological diagnosis (months)		
AML, mean (range)	0.8 (0.03–3.52)	0.8 (0.07–3.84)
MDS, mean (range)	4.9 (0.20–13.63)	4.2 (0.43–14.98)
Disease history, n (%)		
De novo	46 (52.3)	22 (50.0)

Secondary AML/MDS[a]	42 (47.7)	22 (50.0)
ECOG performance status, n (%)		
0	11 (12.5)	3 (6.8)
1	29 (33.0)	18 (40.9)
2	47 (53.4)	23 (52.3)
Not reported	1 (1.1)	0
Cytogenetic risk,* n (%)		
Good/intermediate risk	52 (59.1)	25 (56.8)
Poor risk	36 (40.9)	19 (43.2)
ELN risk stratification for AML,[21] n (%)	N = 78	N = 38
Favorable	5 (6.4)	3 (7.9)
Intermediate-I	27 (34.6)	11 (28.9)
Intermediate-II	21 (26.9)	8 (21.1)
Adverse	25 (32.1)	16 (42.1)
Prognostic factors for MDS,† n (%)	N = 10	N = 6
Good risk	3 (30.0)	2 (33.3)
Intermediate risk	1 (10.0)	3 (50.0)
Poor risk	6 (60.0)	1 (16.7)
MDS IPSS score,[22] n (%)	N = 10	N = 6
0.5–1 (Intermediate-1)	0	2 (33.3)
1.5–2 (Intermediate-2)	4 (40.0)	4 (66.7)
≥2.5 (High)	6 (60.0)	0
Prior therapy with MDS drug,‡ n (%)	N = 88	N = 44
Azacitidine	13 (14.8)	8 (18.2)
Decitabine	2 (2.3)	1 (2.3)

[a] Secondary AML included AML evolving from MDS or other AHD and AML after previous cytotoxic therapy or radiation. Secondary MDS included MDS from prior antecedent hematologic disease (AHD).

* For AML, good/intermediate cytogenetic risk = favorable, intermediate-I, and intermediate-II risk groups; poor cytogenetic risk = adverse risk group.

† MDS risk was assessed by cytogenetics abnormalities that were known at the time the study was initiated; good/intermediate cytogenetic risk = good and intermediate risk groups; poor cytogenetic risk =

poor risk group.

‡ All patients who received prior HMA therapy were considered refractory.

Abbreviations: AML, acute myeloid leukemia; CR, complete remission or complete response; ECOG, Eastern Cooperative Oncology Group; HMA, hypomethylating agents; IPSS, International Prognostic Scoring System; LDAC, low-dose cytarabine; MDS, myelodysplastic syndrome; SD, standard deviation.

Table 2: Proportion of patients with investigator-reported CR, full analysis set

	Glasdegib 100 mg + LDAC N = 88	**LDAC N = 44**
Patients with CR, n (%)	15 (17.0)	1 (2.3)
80% CI*	11.9–22.2	0.0–5.2
95% CI*	9.2–24.9	0.0–6.7
Cytogenetic risk		
Good/intermediate	52	25
Patients with CR, n (%)	10 (19.2)	0 (0.0)
80% exact CI†	12.3–28.1	0.0–8.8
95% CI†	9.6–32.5	0.0–13.7
Poor cytogenetic risk	36	19
Patients with CR, n (%)	5 (13.9)	1 (5.3)
80% exact CI†	6.9–24.2	0.6–19.0
95% CI†	4.7–29.5	0.1–26.0
Combination versus LDAC		
Pearson Chi-square test for all enrolled patients (unstratified)		
P value	0.0142	
CMH test for all enrolled patients stratified by cytogenetics*		
Odds ratio (80% CI)	5.03 (1.59–15.88)	
P value	0.0152	

* Using normal approximation.

† Using exact method based on binomial distribution.

*Good/intermediate and poor cytogenetic risk based on IVRS

Abbreviations: CI, confidence interval; CMH, Cochran-Mantel-Haenszel; CR, complete remission; IVRS, interactive voice response system; LDAC, low-dose cytarabine.

Table 3: Treatment-emergent all-causality adverse events occurring in ≥ 20% of patients in any treatment

MedDRA preferred term*, n (%)	Glasdegib 100 mg + LDAC, N = 84				LDAC, N = 41			
	Grade 1–2	Grade 3–4	Grade 5	Total	Grade 1–2	Grade 3–4	Grade 5	Total
Any AEs	6 (7.1)	54 (64.3)	24 (28.6)	84 (100.0)	1 (2.4)	23 (56.1)	17 (41.5)	41 (100.0)
Anemia	3 (3.6)	35 (41.7)	0	38 (45.2)	2 (4.9)	15 (36.6)	0	17 (41.5)
Febrile neutropenia	0	30 (35.7)	0	30 (35.7)	0	10 (24.4)	0	10 (24.4)
Nausea	28 (33.3)	2 (2.4)	0	30 (35.7)	4 (9.8)	1 (2.4)	0	5 (12.2)
Decreased appetite	25 (29.8)	3 (3.6)	0	28 (33.3)	3 (7.3)	2 (4.9)	0	5 (12.2)
Fatigue	14 (16.7)	12 (14.3)	0	26 (31.0)	6 (14.6)	2 (4.9)	0	8 (19.5)
Thrombocytopenia	0	26 (31.0)	0	26 (31.0)	1 (2.4)	10 (24.4)	0	11 (26.8)
Pneumonia	4 (4.8)	14 (16.7)	6 (7.1)	24 (28.6)	1 (2.4)	6 (14.6)	3 (7.3)	10 (24.4)
Diarrhea	19 (22.6)	4 (4.8)	0	23 (27.4)	8 (19.5)	1 (2.4)	0	9 (22.0)
Pyrexia	21 (25.0)	2 (2.4)	0	23 (27.4)	7 (17.1)	2 (4.9)	0	9 (22.0)
Edema peripheral	22 (26.2)	0	0	22 (26.2)	6 (14.6)	1 (2.4)	0	7 (17.1)
Constipation	20 (23.8)	1 (1.2)	0	21 (25.0)	6 (14.6)	0	0	6 (14.6)
Dysgeusia	21 (25.0)	0	0	21 (25.0)	1 (2.4)	0	0	1 (2.4)

Dyspnoea	15 (17.9)	6 (7.1)	0	21 (25.0)	9 (22.0)	2 (4.9)	0	11 (26.8)
Muscle spasms	15 (17.9)	4 (4.8)	0	19 (22.6)	2 (4.9)	0	0	2 (4.9)
Cough	18 (21.4)	0	0	18 (21.4)	6 (14.6)	1 (2.4)	0	7 (17.1)
Dizziness	17 (20.2)	1 (1.2)	0	18 (21.4)	4 (9.8)	0	0	4 (9.8)
Vomiting	16 (19.0)	2 (2.4)	0	18 (21.4)	3 (7.3)	1 (2.4)	0	4 (9.8)

* MedDRA (version 19.1) coding dictionary applied.

Abbreviations: AE, adverse event; LDAC, low-dose cytarabine; MedDRA, Medical Dictionary for Regulatory Activities.

Supplementary Materials

Methods

Patients

Inclusion Criteria

Patient eligibility should be reviewed and documented by an appropriately qualified member of the investigator's study team before patients are included in the study.

Patients must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Patients with AML or RAEB-2 High-Risk MDS who are newly diagnosed according to the WHO 2008 Classification and previously untreated. Eligible patients with MDS, as well as eligible patients with AML arising from an antecedent hematologic disease (AHD) or MDS, may have had **one** prior regimen with commercially-available agent(s) (e.g., azacitidine or decitabine) for the treatment of their prior hematologic disease. The patients may not have had any prior therapy for their AML.
2. Patients must have a known cytogenetic profile at study entry.
3. AML patients include de-novo AML, AML evolving from MDS or other AHD and AML after previous cytotoxic therapy or radiation (secondary AML).
    - For a diagnosis of AML, a bone marrow blast count of 20% or more is required.
    - For AML defined by cytogenetic aberrations t(8;21), inv(16) or t(16;16) and some cases of erythroleukemia the proportion of bone marrow blasts may be <20%.
    - In AML FAB M6a (erythroid leukemia) ≥20% of non-erythroid cells in the bone marrow must be leukemic blasts and ≥50% of the cells are erythroid precursors.
    - In AML with monocytic or myelomonocytic differentiation, monoblasts and promonocytes, but not abnormal monocytes, are counted as blast equivalents.
4. For a diagnosis of high-risk Myelodysplastic Syndrome RAEB-2 the patient must have 10-19% bone marrow blasts.

5. Age: must be ≥55 years old.
6. ECOG Performance Status 0, 1, or 2.
7. Patients with AML or High-Risk MDS who have **one or more** of the criteria below are considered unfit for intensive chemotherapy (Kantarjian et al, 2006)32 and are eligible:
    - Age ≥75 years.
    - ECOG of 2.
    - Serum creatinine >1.3 mg/dL.
    - Severe cardiac disease (e.g., LVEF <45% by multi-gated acquisition [MUGA] or echocardiography [ECHO] at screening).
8. Adequate Organ Function as defined by the following:
    - Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) ≤3 x upper limit of normal (ULN), or AST and ALT ≤5 x ULN if liver function abnormalities are due to underlying malignancy.
    - Total serum bilirubin ≤2 x ULN (except patients with documented Gilbert's syndrome).
    - Serum creatinine ≤1.5 x ULN or estimated creatinine clearance ≥60 mL/min as calculated using the method standard for the institution.
9. All anti-cancer treatments (unless specified) should be discontinued ≥2 weeks from study entry (defined in Section 6), for example: targeted chemotherapy, radiotherapy, investigational agents, hormones, anagrelide or cytokines.
    - For control of rapidly progressing leukemia, hydroxyurea or leukopheresis may be used before and for up to 1 week after first dose of glasdegib.
    - Patients with controlled CNS leukemia (documented by two consecutive assessments of zero blast count in cerebrospinal fluid), and who are still receiving intra-thecal (IT) therapy at study entry are considered eligible, and will continue to receive IT therapy.
10. Resolved acute effects of any prior therapy to baseline severity or Grade ≤1 CTCAE except for

AEs not constituting a safety risk by investigator judgement.

11. Serum/urine pregnancy test (for females of childbearing potential) that is negative at screening and immediately prior to initiation of treatment (first dose). Male and female patients of childbearing potential must agree to use a highly effective method of contraception throughout the study and for at least 180 days after the last dose of assigned treatment. A patient is of childbearing potential if, in the opinion of the investigator, he/she is biologically capable of having children and is sexually active.
12. Evidence of a personally signed and dated informed consent document indicating that the patient (or a legal representative) has been informed of all pertinent aspects of the study.
13. Willingness and ability to comply with the study scheduled visits, treatment plans, laboratory tests and other procedures.

Patients with leukocytes $\geq 30\times10^9$/L at study entry were excluded; treatment with hydroxyurea or leukapheresis to reduce the leukocyte count below 30×10^9/L prior to enrolment was permitted. Patients with active malignancy were excluded, with the exception of basal cell carcinoma, non-melanoma skin cancer, and cervical carcinoma in situ; other prior or concurrent malignancies were considered on a case-by-case basis. Other exclusion criteria included a recent myocardial infarction, congenital long QT syndrome, Torsades de Pointes, clinically significant ventricular arrhythmias within 6 months of study entry, or corrected QT (QTc) interval > 470 ms using Fridericia's formula (QTcF).

Assessments

Samples for bone marrow evaluation were collected at screening, on Cycle 3 Day 1 and every third cycle, within 14 days of achieving initial hematologic recovery in the peripheral blood (defined as absolute neutrophil count >1000/μL and platelets ≥100,000/μL), end of treatment, and at the investigator discretion (±7 days of nominal time).

Blood samples for PK analysis of glasdegib were collected on Cycle 1 Day 1 at pre-dose and 1

and 4 hours post-dose; Cycle 1 Day 10 at pre-dose and 1, 2, 4, and 6 hours post-dose; and Cycles 2, 3, 4, and 5 on Day 1 at pre-dose, 1 and 4 hr post-dose.

Calibration standard responses were linear over the range of 0.2 ng/mL to 200 ng/mL for glasdegib, using a l/concentration^2-weighted linear regression. The lower limit of quantification (LLOQ) for glasdegib was 0.2 ng/mL. Samples with plasma glasdegib concentrations below the LLOQ were reported as less than the LLOQ.

The inter-batch assay accuracy, expressed as percent relative error of the mean glasdegib quality control (QC) sample concentrations, ranged from –8.1% to 1.3%. Inter-batch assay precision, expressed as percent coefficient of variation (%CV) of the estimated glasdegib concentrations of QC samples, was ≤8.5%.

The pharmacokinetic (PK) parameters were calculated using noncompartmental analysis and included: maximum observed plasma concentration (C_{max}), time to C_{max}, area under the plasma concentration–time curve from time 0 to tau (tau = dosing interval of 24 hr), average plasma concentration at steady-state, and predose plasma concentration.

Treatment duration was calculated as the last dosing date of study drug minus Cycle 1 Day 1 plus 1 day, where last dosing date was the last non-zero dose date and it included missed doses on unknown dates. Time of treatment exposure of glasdegib was calculated as the last dosing date of study drug minus Cycle 1 Day 1 plus 1 day, where last dosing date was the last non-zero dose date and it excluded days with total dose administer of 0 mg.

Relative dose intensity was calculated as follows: relative dose intensity while on treatment = {actual total dose received/weeks from treatment start to end of treatment}/{planned intensity}, planned intensity = {initial planned dose/planned number of weeks in a cycle}, and actual cycle intensity = {actual received cycle dose/number of weeks in the cycle including delays}.

DNA samples extracted from peripheral blood or bone marrow were analyzed using next-generation DNA sequencing validated to Good Clinical Practice guidelines of a panel of 12 genes performed using the Illumina® MiSeq instrument (San Diego, CA, USA). In a secondary assay, an

amplicon-based approach was used to further characterize the *FLT3* gene for the presence of internal tandem duplication (ITD) mutations. Whole blood samples from serial blood draws were analyzed for gene expression using TaqMan Low-Density Microarrays (TLDA). These TLDA cards included 21 target genes implicated in Smoothened pathway signaling and/or AML pathobiology, 2 endogenous control reference genes (*GUSB* and *TBP*), and 1 manufacturing control gene (*GAPDH*). The subset of time-points prioritized for gene expression and associated statistical analysis were short-term where blast counts were generally not substantially different compared with baseline, or were at end of treatment when blast counts had often rebounded.

Statistical Analyses

A total of 92 overall survival (OS) events were needed to provide 80% power to detect a difference between the two arms. This was based on 2:1 randomization, a planned accrual period of approximately 13 months, a follow-up period of approximately 6 months, a one-sided log-rank test with alpha = 0.1 (type I error), and one futility analysis when 46 OS events were observed (50% information, rho[1] beta spending function).

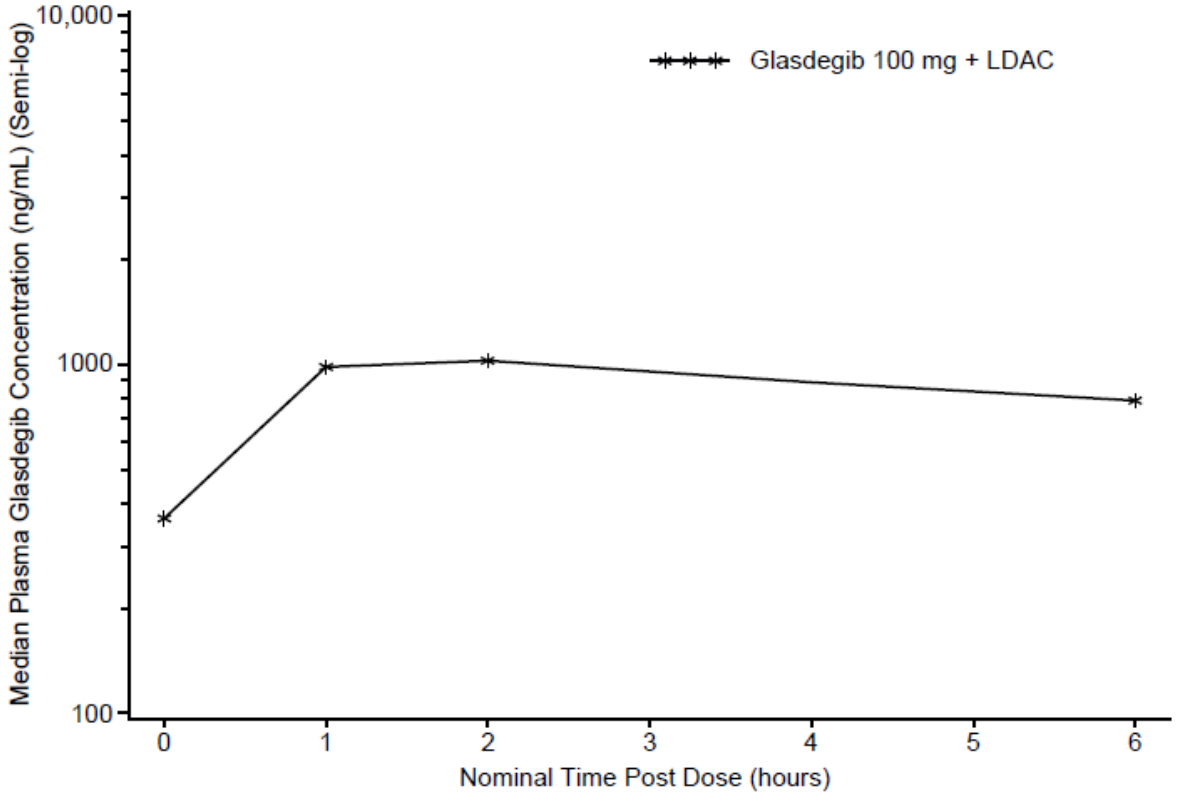
Results

Findings of RNA Biomarker Analysis

RNA biomarkers were analyzed in 64 patients (47 and 17 patients in the glasdegib/LDAC and LDAC arms, respectively). Of the 21 mRNAs evaluated, several in the glasdegib/LDAC arm exhibited significant changes from baseline to end of treatment, including *CCND1* (median 60% lower than baseline, $P = .0448$), *CCND2* (median 30% lower than baseline, $P = .0004$), and *SMO* (median 60% lower than baseline, $P = .0094$). In the glasdegib/LDAC arm, response was associated with lower baseline *FOXM1* mRNA expression (median 50% of non-responders; $P = .0258$) and higher baseline *PTCH1* mRNA expression (median 2-fold higher than non-responders; $P = .0002$). Higher Cycle 1 Day 1 1h post-dose *MYCN* mRNA expression was associated with response (median ratio to baseline of 1.6 for

responders relative to 0.5 for non-responders, $P = .0312$). Expression of mRNAs encoding the GLI1 and GLI2 transcription factors (Hedgehog pathway dependent transcripts) did not prove evaluable in almost all blood samples.

Supplemental Figure S1: Median glasdegib plasma concentration–time profile on cycle 1 day 10, semi-log



LDAC, low-dose cytarabine

Supplementary Table S1: Follow-up systemic therapies in all patients who received glasdegib 100 mg + IDAC and LDAC only

	Glasdegib 100 mg + LDAC	**LDAC**
Total patients, n	84	41
With follow-up systemic therapies, n (%)	37 (44.0)	15 (36.6)
Transplant	1 (1.2)	0
Chemotherapy	34 (40.5)	14 (34.1)
Biologic	0	0
Tyrosine kinase inhibitor	0	0
Investigational	2 (2.4)	0
Other	0	1 (2.4)

Supplementary Table S2: Deaths within 30 days and 60 days of treatment initiation

	Total				AML				MDS			
	Glasdegib 100 mg + LDAC, N = 84		LDAC N = 41		Glasdegib 100 mg + LDAC, N = 75		LDAC N = 36		Glasdegib 100 mg + LDAC, N = 9		LDAC N = 5	
	n (%)	80% CI	n (%)	80% CI	n (%)	80% CI	n (%)	80% CI	n (%)	80% CI	n (%)	80% CI
Deaths within 30 days	5 (6.0)	2.9–10.8	5 (12.2)	6.1–21.5	5 (6.7)	3.3–12.0	5 (13.9)	6.9–24.2	0	0	0	0
Cause of death										0		0
Disease under study	4 (4.8)		4 (9.8)		4 (5.3)		4 (11.1)			0		0
Other	1 (1.2)		4 (9.8)		1 (1.3)		4 (11.1)			0		0
Deaths within 60 days	10 (11.9)	7.5–17.7	13 (31.7)	22.1–42.8	8 (10.7)	6.3–16.7	13 (36.1)	25.3–48.1	2 (22.2)	6.1–49.0	0	0
Cause of death												
Disease under study	9 (10.7)		12 (29.3)		7 (9.3)		12 (33.3)		2 (22.2)		0	
Other	3 (3.6)		5 (12.2)		2 (2.7)		5 (13.9)		1 (11.1)		0	

Supplementary Table S3: All-causality treatment-emergent serious adverse events by MedDRA preferred term in ≥2 patients (all cycles, safety analysis set)

	Glasdegib 100 mg + LDAC, N = 84					LDAC Alone, N = 41			
MedDRA preferred term	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**	**Total**	**Grade 3**	**Grade 4**	**Grade 5**	**Total**
Any SAEs	2 (2.4)	28 (33.3)	12 (14.3)	24 (28.6)	66 (78.6)	9 (22.0)	6 (14.6)	17 (41.5)	32 (78.0)
Febrile neutropenia	0	20 (23.8)	4 (4.8)	0	24 (28.6)	5 (12.2)	2 (4.9)	0	7 (17.1)
Pneumonia	1 (1.2)	10 (11.9)	2 (2.4)	6 (7.1)*	19 (22.6)	2 (4.9)	2 (4.9)	3 (7.3)	7 (17.1)
Disease progression	0	0	0	8 (9.5)	8 (9.5)	0	0	5 (12.2)	5 (12.2)
Anemia	0	4 (4.8)	2 (2.4)	0	6 (7.1)				
Syncope	0	4 (4.8)	0	0	4 (4.8)				
Acute kidney injury	1 (1.2)	2 (2.4)	0	0	3 (3.6)				
Fatigue	2 (2.4)	1 (1.2)	0	0	3 (3.6)				
Hemorrhage intracranial	0	0	2 (2.4)	1 (1.2)	3 (3.6)				
Pyrexia	2 (2.4)	1 (1.2)	0	0	3 (3.6)				
Sepsis	0	0	3 (3.6)	0	3 (3.6)	0	1 (2.4)	4 (9.8)†	5 (12.2)
Cardiac arrest	0	0	1 (1.2)	1 (1.2)	2 (2.4)				
Cardiac failure	0	0	2 (2.4)	0	2 (2.4)				
Fall	1 (1.2)	1 (1.2)	0	0	2 (2.4)				
Gastrointestinal hemorrhage	0	1 (1.2)	1 (1.2)	0	2 (2.4)				
Hyponatremia	0	1 (1.2)	1 (1.2)	0	2 (2.4)				
Muscular weakness	0	2 (2.4)	0	0	2 (2.4)				
Myocardial infarction	0	0	1 (1.2)	1 (1.2)	2 (2.4)				
Septic shock	0	0	1 (1.2)	1 (1.2)	2 (2.4)				
Sudden death	0	0	0	2 (2.4)	2 (2.4)				
Pancytopenia						2 (4.9)	0	0	2 (4.9)

Values are n (%).

Treatment-emergent adverse events (AEs) were defined as within 28 days of last dose of study treatment and graded in accordance with National Cancer Institute CTCAE version 4.03. Grade 5 is death related to AE. The type of Grade 5 events were characteristic of patients with acute myeloid malignancies, elderly patients, and chemotherapy treatment. No Grade 1 AEs were reported in ≥2 patients in either treatment arm. No Grade 2 AEs were reported in ≥2 patients in the LDAC arm.

* One (1.2%) was considered as treatment-related Grade 5 AE.

† One (2.4%) was treatment-related Grade 5 AE.

Supplementary Table S4: Investigator-reported best overall response for patients with AML, full analysis set

	Glasdegib 100 mg + LDAC N = 78		LDAC N = 38	
	n (%)	**80% CI**	**n (%)**	**80% CI**
Objective response*				
Disease status				
CR	14 (17.9)	12.4–24.8	1 (2.6)	0.3–9.9
CRi	5 (6.4)	3.2–11.6	1 (2.6)	0.3–9.9
MLFS	2 (2.6)	0.7–6.7	0 (0.0)	0.0–5.9
Not evaluable†	24 (30.8)	23.9–38.4	16 (42.1)	31.1–53.8
ORR (CR+CRi+MLFS)‡	21 (26.9)	20.5–33.4	2 (5.3)	0.6–9.9

* Using exact method based on binomial distribution and CIs are expressed in percentages.

† In addition to the seven patients who were randomized but not treated, the majority of patients not evaluable for disease response in both arms were due to AE or patient died prior to on-study bone marrow evaluation.

‡ Using normal approximation for further endpoints of interest and CIs are expressed in percentages.

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; CRi, CR with incomplete blood count recovery; LDAC, low dose cytarabine; MLFS, morphologic leukemia-free state; N, all treated patients; ORR, overall response rate.

Supplementary Table S5: Investigator-reported best overall response for patients with MDS,[1] full analysis set

	Glasdegib 100 mg + LDAC N = 10		LDAC N = 6	
	n (%)	**80% CI**	**n (%)**	**80% CI**
Objective response*				
Disease status				
CR/Unconfirmed CR	1 (10.0)	1.0–33.7	0 (0.0)	0.0–31.9
PR/Unconfirmed PR	0 (0.0)	0.0–20.6	0 (0.0)	0.0–31.9
mCR/Unconfirmed mCR	1 (10.0)	1.0–33.7	0 (0.0)	0.0–31.9
Not evaluable†	2 (20.0)	5.5–45.0	1 (16.7)	1.7–51.0
ORR (CR+mCR)‡	2 (20.0)	5.5–45.0	0 (0.0)	0.0–31.9

* Using exact method based on binomial distribution and CIs are expressed in percentages.

† On the glasdegib + LDAC arm, 1 patient did not receive study treatments and the other patient had adverse event prior to bone marrow evaluation; on the LDAC arm, the patient did not receive study treatment.

‡ Using normal approximation for further endpoints of interest and CIs are expressed in percentages.

Abbreviations: CI, confidence interval; CR, complete remission; LDAC, low dose cytarabine; mCR, marrow complete remission; MDS, myelodysplastic syndrome; N, all treated patients; ORR, overall response rate; PR, partial remission.

Reference:

1. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108:419-25.

Supplementary Table S6: Patients who were not evaluable for disease response

	Glasdegib 100 mg + LDAC (N = 26)	LDAC (N = 17)	Total (N = 43)
Reasons for not being evaluable for bone marrow disease response, n (%)			
AE leading to study termination prior to disease assessment	9 (34.6)	5 (29.4)	14 (32.5)
Patient died prior to disease assessment	3 (11.5)	6 (35.3)	9 (20.9)
Never started treatment	4 (15.4)	3 (17.6)	7 (16.3)
Insufficient clinical response (based on peripheral blood only, no bone marrow performed)	4 (15.4)	2 (11.8)	6 (14)
Patient refused prior to disease assessment (withdrew consent, local treatment, no further treatment, unwilling to comply)	5 (19.2)	1 (5.9)	6 (14)
Global deterioration of health	1 (3.9)	0	1 (2.3)

Abbreviations: AE, adverse event; AML, acute myeloid leukemia; LDAC, low dose cytarabine; MDS, myelodysplastic syndrome.

Appendix Table 6: Glasdegib plasma pharmacokinetic parameters for glasdegib 100 mg + IDAC arm on cycle 1 day 10

Parameter, Units	Dose Compliant, Non-CYP3A4 (N = 41)*	Dose Compliant (N = 61)†
C_{max}, ng/mL	1252 (44)	1343 (47)
T_{max}, hr	1.7 (0.67–5.8)	2.0 (0.67–6.3)
AUC_{tau}, ng.hr/mL‡	17210 (54)	19170 (61)
C_{avg}, ng/mL	718 (54)	799 (61)
C_{trough}, ng/mL	427 (80)	483 (88)

Values are geometric mean (% geometric CV) for all, except median (range) for T_{max}.

* n = 36 for C_{trough}; n = 37 for AUC_{tau} and C_{avg}.

† n = 55 for C_{trough}; n = 56 for AUC_{tau} and C_{avg}.

‡ For AUC_{tau}, tau = 24 hr. For AUC_{tau}, the pre-dose concentration was also designated as the 24-hr post-dose sample to estimate AUC_{tau}, using assumption of steady state.

Abbreviations: %CV, percent coefficient of variation; CYP, cytochrome P450; LDAC, low-dose cytarabine.

Appendix Table 7: Treatment-related* all causality adverse events occurring in ≥ 10% of patients in any treatment arm

MedDRA preferred term,* n (%)	Glasdegib 100 mg + LDAC, N = 84			LDAC, N = 41		
	Grade 1-2	Grade 3-5	Total	Grade 1-2	Grade 3-5	Total
Any adverse event	13 (15.5)	55 (65.5)	68 (81.0)	10 (24.4)	14 (34.1)	24 (58.5)
Anaemia	4 (4.8)	22 (26.2)	26 (31.0)	1 (2.4)	5 (12.2)	6 (14.6)
Nausea	23 (27.4)	1 (1.2)	24 (28.6)	1 (2.4)	0	1 (2.4)
Decreased appetite	19 (22.6)	2 (2.4)	21 (25.0)	1 (2.4)	1 (2.4)	2 (4.9)
Thrombocytopenia	0	20 (23.8)	20 (23.8)	0	5 (12.2)	5 (12.2)
Dysgeusia	19 (22.6)	0	19 (22.6)	0	0	0
Fatigue	10 (11.9)	9 (10.7)	19 (22.6)	3 (7.3)	1 (2.4)	4 (9.8)
Muscle spasms	13 (15.5)	4 (4.8)	17 (20.2)	0	0	0
Diarrhea	11 (13.1)	3 (3.6)	14 (16.7)	1 (2.4)	0	1 (2.4)
Vomiting	12 (14.3)	2 (2.4)	14 (16.7)	3 (7.3)	0	3 (7.3)
Platelet count decreased	1 (1.2)	12 (14.3)	13 (15.5)	0	1 (2.4)	1 (2.4)
Febrile neutropenia	0	12 (14.3)	12 (14.3)	0	3 (7.3)	3 (7.3)
Weight decreased	12 (14.3)	0	12 (14.3)	0	0	0
White blood cell count decreased	1 (1.2)	10 (11.9)	11 (13.1)	1 (2.4)	0	1 (2.4)
Constipation	10 (11.9)	0	10 (11.9)	3 (7.3)	0	3 (7.3)
Dyspnoea	8 (9.5)	2 (2.4)	10 (11.9)	1 (2.4)	0	1 (2.4)
Neutrophil count decreased	1 (1.2)	9 (10.7)	10 (11.9)	0	1 (2.4)	1 (2.4)
Alopecia	9 (10.7)	0	9 (10.7)	0	0	0
Neutropenia	3 (3.6)	6 (7.1)	9 (10.7)	0	4 (9.8)	4 (9.8)

*Adverse events as related to either LDAC and/or glasdegib.

Abbreviations: LDAC, low-dose cytarabine; MedDRA, Medical Dictionary for Regulatory Activities.

Appendix Table 8: Summary and analysis of baseline gene mutation frequency in responding patients, by treatment arm

Glasdegib 100 mg + LDAC N = 61		**LDAC N = 27**	
Mutation	**ORR, n (%)**	**Mutation**	**ORR, n (%)**
CEBPA, n = 8	3 (38)	CEBPA, n = 3	0
DNMT3A, n = 15	2 (13)	*DNMT3A*, n = 6	0
FLT3, n = 5	1 (20)	*FLT3*, n = 0	0
FLT3-ITD, n = 3	1 (33)	*FLT3-ITD*, n = 2	0
IDH1, n = 10	5 (50)	*IDH1*, n = 2	0
IDH2, n = 12	2 (17)	*IDH2*, n = 5	0
KIT, n = 3	1 (33)	*KIT*, n = 1	0
KRAS, n = 2	0	*KRAS*, n = 2	0
NPM1, n = 5	2 (40)	*NPM1*, n = 1	0
NRAS, n = 5	1 (20)	*NRAS*, n = 3	0
RUNX1, n = 28	10 (36)	*RUNX1*, n = 7	0
TET2, n = 15	7 (47)	*TET2*, n = 9	1 (11)
WT1, n = 3	1 (33)	*WT1*, n = 1	0

The analysis population included patients with available sequencing results who were evaluable for response. Baseline mutational status determined from the combined results from evaluable bone marrow and/or whole blood samples. Mutational status assessed using next-generation sequencing (augmented by an amplicon-based assay in the case of FLT3-ITD mutations).

Statistical significance in comparison of responders with non-responders was determined using Fisher's exact test. $P > .30$ for all evaluable comparisons.

For AML, investigator-reported ORR = CR+CRi+MLFS; for MDS investigator-reported ORR = CR+mCR.

Abbreviations: AML, acute myeloid leukaemia; CR, complete remission; CRi, complete remission with incomplete blood count recovery; LDAC, low-dose cytarabine; mCR, marrow complete remission; MDS, myelodysplastic syndrome; MLFS, morphologic leukaemia-free state; ORR, overall response rate; SD, standard deviation.